SEQUENCE LISTING

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<120> ANTIBODY-MEDIATED DOWN-REGULATION OF PLANT PROTEINS

<130> 50447

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<150> US 60/093,587
<151> 1998-07-21

<160> 56

<170> PatentIn Ver. 2.0

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<213> Zea mays

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<222> (1)..(276)

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Glu Glu Asn Arg His Gly Asp Leu Leu Asn Lys Tyr Met Tyr Leu Thr
1 5 10 15

ggg agg gtg gat atg agg cag att gag aag aca att cag tat ctt att 96

Another down regulation approach involves the use of ribozyme technology as described by Hasselhoff and Gerlach, (1988) Nature 342:76-79. Ribozyme technology, like antisense methodologies, also works at the RNA translational level and involves making catalytic RNA molecules which bind to and cleave the mRNA of interest. Ribozymes have recently been demonstrated as an effective method for the down-regulation of plant proteins as described in WO97/10328 to Ribozyme Pharmaceuticals and Dow AgroSciences, LLC, formally DowElanco.

Co-suppression, as described by Seymour et al., (1993, Plant Mol. Biol. 23:1-9) is another approach applicable for down-regulation of plant gene expression. At present, the precise mechanism of down-regulation via co-suppression is not known. However, it has been used extensively to produce transgenic plants having modified gene expression levels as described in Brusslan et al., (1993) Plant Cell 5:667-677; Vaucheret et al., (1995) Mol. Gen. Genet. 248:311-317; and Jorgensen et al., (196) Plant Mol. Biol. 31:957-973.

As disclosed herein, Applicants have invented an alternative approach to down-regulate proteins in plants relying on the use of monoclonal antibodies (MAb) and functional fragments thereof, such as single chain antibodies (SCAb) that specifically recognize and bind transit peptides. As a result, steady-state levels of corresponding passenger proteins can be reduced. The proposed approach is further exemplified, as shown in the non-limiting examples, through down-regulation of maize stearoyl-ACP Δ-9 desaturase.

In many situations it will be desirable to modify an existing trait of a plant cell rather than introduce a new trait. Thus, one may wish to modify the overall activity levels of a particular enzyme, provide for preferential accumulation of one allele as compared to another, one isozyme as compared to another or the like. In other instances, one may only wish to reduce the amount of expression of a protein encoded by a gene

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atcccaatct cgcgagggca agcagcaggg tctgcggcgg cggcggcggc cgcgcttccg 120

gctccccttc ccattggcct ccacg atg gag ctc cgc ctc aac gac gtc gcg   172
                            Met Ala Leu Arg Leu Asn Asp Val Ala
                             -30                 -25

ctc tgc ctc tcc ccg ccg ctc gcc gcc cgc cgc cgc cgc cgc agc agc   220
Leu Cys Leu Ser Pro Pro Leu Ala Ala Arg Arg Arg Arg Arg Ser Ser
        -20                 -15                 -10

ggc agg ttc gtc gcc gtc gcc tcc atg acg tcc gcc gtc tcc acc aag   268
Gly Arg Phe Val Ala Val Ala Ser Met Thr Ser Ala Val Ser Thr Lys
     -5                 -1   1                   5                  10

gtc gag aat aag aag cca ttt gct cct cca agg gag gta cat gtc cag   316
Val Glu Asn Lys Lys Pro Phe Ala Pro Pro Arg Glu Val His Val Gln
                15                  20                  25

gtt aca cat tca atg cca cct cac aag att gaa att ttc aag tcg ctt   364
Val Thr His Ser Met Pro Pro His Lys Ile Glu Ile Phe Lys Ser Leu
            30                  35                  40

gat gat tgg gct aga gat aat atc ctg acg cat ctc aag cca gtc gag   412
Asp Asp Trp Ala Arg Asp Asn Ile Leu Thr His Leu Lys Pro Val Glu
        45                  50                  55

aag tgt tgg cag cca cag gat ttc ctc ccg gac cca gca tct gaa gga   460
Lys Cys Trp Gln Pro Gln Asp Phe Leu Pro Asp Pro Ala Ser Glu Gly
    60                  65                  70

ttt cat gat gaa gtt aag gag ctc aga gaa cgt gcc aag gaa atc cct   508
Phe His Asp Glu Val Lys Glu Leu Arg Glu Arg Ala Lys Glu Ile Pro
75                  80                  85                  90

gat gat tat ttt gtt tgt ttg gtg gga gac atg att acc gag gaa gct   556
Asp Asp Tyr Phe Val Cys Leu Val Gly Asp Met Ile Thr Glu Glu Ala
                95                  100                 105

cta cca aca tac cag act atg ctt aac acc ctc gac ggt gtc aga gat   604
Leu Pro Thr Tyr Gln Thr Met Leu Asn Thr Leu Asp Gly Val Arg Asp
            110                 115                 120

gag aca ggt gca agc ccc act gcc tgg gct gtt tgg acg agg gca tgg   652
Glu Thr Gly Ala Ser Pro Thr Ala Trp Ala Val Trp Thr Arg Ala Trp
        125                 130                 135

act gct gag gag aac agg cat ggt gat ctg ctc aac aag tat atg tac   700
Thr Ala Glu Glu Asn Arg His Gly Asp Leu Leu Asn Lys Tyr Met Tyr
    140                 145                 150

ctc act ggg agg gtg gat atg agg cag att gag aag aca att cag tat   748
Leu Thr Gly Arg Val Asp Met Arg Gln Ile Glu Lys Thr Ile Gln Tyr
155                 160                 165                 170

ctt att ggc tct gga atg gat cct agg act gag aat aat cct tat ctt   796
Leu Ile Gly Ser Gly Met Asp Pro Arg Thr Glu Asn Asn Pro Tyr Leu
                175                 180                 185

ggt ttc atc tac acc tcc ttc caa gag cgg gcg acc ttc atc tca cac   844
Gly Phe Ile Tyr Thr Ser Phe Gln Glu Arg Ala Thr Phe Ile Ser His
            190                 195                 200
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antibodies and single chain antibodies (SCAb) immunologically reactive to said transit peptides in combination with promoter regulatory elements and use of said chimeric genes within a plant cell. Expression of said genes can result in functional antibody proteins which bind to transit peptides and thus lowers steady state levels of passenger proteins in a cell organelle.

Yet an additional aspect of the present invention is transformation of plant species disclosed herein with said chimeric genes.

Other aspects, embodiments, advantages, and features of the present invention will become apparent from the following specification.

Detailed Description of the Invention

The present invention relates to methods and compositions for obtaining transgenic plants having altered steady state passenger protein levels due to expression of antibodies being immunologically reactive to transit peptides. The following phrases and terms are defined below:

By "altered steady state" is meant that the total level of a particular passenger plant protein having a transit peptide in a modified plant is different from that of a normal or non-modified plant under similar conditions. Decreased steady state levels can be achieved by expressing antibodies immunologically reactive to said transit peptide.

By "antibody" is meant a protein molecule having functional activity comprising two identical polypeptide chains of about 600 amino acid residues (usually referred to as heavy chains, H) covalently attached to each other by disulfide bonds, and two identical shorter polypeptide chains of about 220 amino acid residues (usually referred

Ala Ala Arg Arg Arg Arg Arg Ser Ser Gly Arg Phe Val Ala Val Ala
-15 -10 -5 -1 1

Ser Met Thr Ser Ala Val Ser Thr Lys Val Glu Asn Lys Lys Pro Phe
5 10 15

Ala Pro Pro Arg Glu Val His Val Gln Val Thr His Ser Met Pro Pro
20 25 30

His Lys Ile Glu Ile Phe Lys Ser Leu Asp Asp Trp Ala Arg Asp Asn
35 40 45

Ile Leu Thr His Leu Lys Pro Val Glu Lys Cys Trp Gln Pro Gln Asp
50 55 60 65

Phe Leu Pro Asp Pro Ala Ser Glu Gly Phe His Asp Glu Val Lys Glu
70 75 80

Leu Arg Glu Arg Ala Lys Glu Ile Pro Asp Asp Tyr Phe Val Cys Leu
85 90 95

Val Gly Asp Met Ile Thr Glu Glu Ala Leu Pro Thr Tyr Gln Thr Met
100 105 110

Leu Asn Thr Leu Asp Gly Val Arg Asp Glu Thr Gly Ala Ser Pro Thr
115 120 125

Ala Trp Ala Val Trp Thr Arg Ala Trp Thr Ala Glu Glu Asn Arg His
130 135 140 145

Gly Asp Leu Leu Asn Lys Tyr Met Tyr Leu Thr Gly Arg Val Asp Met
150 155 160

Arg Gln Ile Glu Lys Thr Ile Gln Tyr Leu Ile Gly Ser Gly Met Asp
165 170 175

Pro Arg Thr Glu Asn Asn Pro Tyr Leu Gly Phe Ile Tyr Thr Ser Phe
180 185 190

Gln Glu Arg Ala Thr Phe Ile Ser His Gly Asn Thr Ala Arg His Ala
195 200 205

Lys Asp Phe Gly Asp Leu Lys Leu Ala Gln Ile Cys Gly Ile Ile Ala
210 215 220 225

Ser Asp Glu Lys Arg His Glu Thr Ala Tyr Thr Lys Ile Val Glu Lys
230 235 240

Leu Phe Glu Ile Asp Pro Asp Gly Thr Val Val Ala Leu Ala Asp Met
245 250 255

Met Arg Lys Lys Ile Ser Met Pro Ala His Leu Met Phe Asp Gly Gln
260 265 270

Asp Asp Lys Leu Phe Glu His Phe Ser Met Val Ala Gln Arg Leu Gly
275 280 285

Val Tyr Thr Ala Arg Asp Tyr Ala Asp Ile Leu Glu Phe Leu Val Asp
290 295 300 305

Arg Trp Lys Val Ala Ser Leu Thr Gly Leu Ser Gly Glu Gly Asn Lys

By "functional" fragments, and "functional antibodies" is meant that the antibody or fragments thereof retain their ability to bind epitopes used in the immunization process and production of said antibody.

By "gene" is meant to include all genetic material involved in protein expression including chimeric DNA constructions, genes, plant genes and portions thereof.

By "genome" is meant genetic material contained in each cell of an organism and/or virus.

By "indigenous" gene is meant the gene encoding the precursor protein, passenger protein, or transit peptide as found in the native organism. Typically, the transit peptide and passenger protein are found together in the source from which the gene was isolated, although not required to be within the scope of the invention. A non-limiting example of an indigenous gene would be the gene encoding the precursor protein maize Δ-9 desaturase, said gene being found in maize.

By "inducible promoter" is meant promoter elements which are responsible for expression of genes in response to a specific signal, such as: physical stimuli (heat shock genes); light (RUBP carboxylase); hormone (Em); metabolites and stress.

By "mature protein" or "passenger protein" is meant the protein which is found after processing and passing into an organelle. Passenger proteins are originally made in a precursor form that includes a transit peptide and said passenger protein. Upon entry into an organelle, the transit peptide portion is cleaved, thus leaving the "passenger" or "mature" protein. Passenger protein are the proteins typically obtained upon purification from an homogenate, as described herein. The sequence of passenger proteins can be determined as described herein. Passenger proteins can be indigenous in that they are naturally found in the organelle of interest. Passenger proteins can also be non-indigenous

<211> 1107
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<220>
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<222> (4)..(1092)

<400> 11

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    Met Ala Thr Met Thr Ser Ala Val Ser Thr Lys Val Glu Asn Lys
      1               5                  10                  15

aag cca ttt gct cct cca agg gag gca cat gtc cag gtt aca cat tca   96
Lys Pro Phe Ala Pro Pro Arg Glu Val His Val Gln Val Thr His Ser
                20                  25                  30

atg cca cct cac aag atc gaa att ttc aag tcg ctt gat gat tgg gct  144
Met Pro Pro His Lys Ile Glu Ile Phe Lys Ser Leu Asp Asp Trp Ala
            35                  40                  45

aga gat aat atc ttg acg cat ctc aag cca gtc gag aag tgt tgg cag  192
Arg Asp Asn Ile Leu Thr His Leu Lys Pro Val Glu Lys Cys Trp Gln
        50                  55                  60

cca cag gat ttc ctc ccg gac cca gca tct gaa gga ttt cat gat gaa  240
Pro Gln Asp Phe Leu Pro Asp Pro Ala Ser Glu Gly Phe His Asp Glu
    65                  70                  75

gtt aag gag ctc aga gaa cgt gcc aag gaa atc cct gat gat tat ttt  288
Val Lys Glu Leu Arg Glu Arg Ala Lys Glu Ile Pro Asp Asp Tyr Phe
80                  85                  90                  95

gtt tgt ttg gtg gga gac atg att acc gag gaa gct cta cca aca tac  336
Val Cys Leu Val Gly Asp Met Ile Thr Glu Glu Ala Leu Pro Thr Tyr
                100                 105                 110

cag act atg ctt aac acc ctc gac ggt gtc aga gat gag aca ggt gca  384
Gln Thr Met Leu Asn Thr Leu Asp Gly Val Arg Asp Glu Thr Gly Ala
            115                 120                 125

agc ccc act gcc tgg gct gtt tgg acg agg gca tgg act gct gag gag  432
Ser Pro Thr Ala Trp Ala Val Trp Thr Arg Ala Trp Thr Ala Glu Glu
        130                 135                 140

aac agg cat ggt gat ctg ctc aac aag tat atg tac ctc act ggg agg  480
Asn Arg His Gly Asp Leu Leu Asn Lys Tyr Met Tyr Leu Thr Gly Arg
    145                 150                 155

gtg gat atg agg cag att gag aag aca att cag tat ctt att ggc tct  528
Val Asp Met Arg Gln Ile Glu Lys Thr Ile Gln Tyr Leu Ile Gly Ser
160                 165                 170                 175

gga atg gat cct agg act gag aat aat cct tat ctt ggt ttc atc tac  576
Gly Met Asp Pro Arg Thr Glu Asn Asn Pro Tyr Leu Gly Phe Ile Tyr
                180                 185                 190

acc tcc ttc caa gag cgg gcg acc ttc atc tca cac ggg aac act gct  624
Thr Ser Phe Gln Glu Arg Ala Thr Phe Ile Ser His Gly Asn Thr Ala
            195                 200                 205
```

gene. Promoter sequences provide the recognition for RNA polymerase and other transcriptional factors required for efficient transcription. Promoter regulatory elements are also meant to include constitutive, tissue-specific, developmental-specific, inducible promoters and the like. Promoter regulatory elements may also include certain enhancer sequence elements that improve transcriptional efficiency. In addition, promoters and promoter regulatory elements, as used herein, are meant to include subgenomic promoters found in +sense, single-stranded RNA viruses such as tobacco mosaic virus and the like.

By "tissue-specific" promoter is meant promoter elements responsible for gene expression in specific cell or tissue types, such as the leaves or seeds (i.e., zein, oleosin, napin, ACP, globulin and like).

By "transgenic plant" is meant a plant expressing a chimeric gene introduced through transformation efforts.

By "transit peptide" or "signal peptide" is meant those amino acids that direct a passenger protein to a particular organelle as defined herein. It is within the scope of this invention that the transit peptide can be either indigenous or non-indigenous to the plant cell of interest. In addition, as further described herein, the transit peptide can be either homologous or heterologous to the passenger protein of interest in that it is natively found with said passenger protein as isolated from nature (homologous) or not (heterologous).

The present invention is directed to down regulation of organeller proteins by expression of antibodies or functional fragments thereof that are immunologically reactive to transit peptides. Down regulation can result in decreased steady state levels of the passenger proteins or enzymatic activity levels of said passenger proteins. Passenger protein may be homologous or heterologous with respect to the transit peptide. Furthermore, transit peptides may be homologous or

Lys Glu Leu Arg Glu Arg Ala Lys Glu Ile Pro Asp Asp Tyr Phe Val
85 90 95

Cys Leu Val Gly Asp Met Ile Thr Glu Glu Ala Leu Pro Thr Tyr Gln
100 105 110

Thr Met Leu Asn Thr Leu Asp Gly Val Arg Asp Glu Thr Gly Ala Ser
115 120 125

Pro Thr Ala Trp Ala Val Trp Thr Arg Ala Trp Thr Ala Glu Glu Asn
130 135 140

Arg His Gly Asp Leu Leu Asn Lys Tyr Met Tyr Leu Thr Gly Arg Val
145 150 155 160

Asp Met Arg Gln Ile Glu Lys Thr Ile Gln Tyr Leu Ile Gly Ser Gly
165 170 175

Met Asp Pro Arg Thr Glu Asn Asn Pro Tyr Leu Gly Phe Ile Tyr Thr
180 185 190

Ser Phe Gln Glu Arg Ala Thr Phe Ile Ser His Gly Asn Thr Ala Arg
195 200 205

His Ala Lys Asp Phe Gly Asp Leu Lys Leu Ala Gln Ile Cys Gly Ile
210 215 220

Ile Ala Ser Asp Glu Lys Arg His Glu Thr Ala Tyr Thr Lys Ile Val
225 230 235 240

Glu Lys Leu Phe Glu Ile Asp Pro Asp Gly Thr Val Val Ala Leu Ala
245 250 255

Asp Met Met Arg Lys Lys Ile Ser Met Pro Ala His Leu Met Phe Asp
260 265 270

Gly Gln Asp Asp Lys Leu Phe Glu His Phe Ser Met Val Ala Gln Arg
275 280 285

Leu Gly Val Tyr Thr Ala Arg Asp Tyr Ala Asp Ile Leu Glu Phe Leu
290 295 300

Val Asp Arg Trp Lys Val Ala Ser Leu Thr Gly Leu Ser Gly Glu Gly
305 310 315 320

Asn Lys Ala Gln Asp Tyr Leu Cys Thr Leu Ala Ser Arg Ile Arg Arg
325 330 335

Leu Glu Glu Arg Ala Gln Ser Arg Ala Lys Lys Ala Gly Thr Leu Pro
340 345 350

Phe Ser Trp Val Tyr Gly Arg Asp Val Gln Leu
355 360

<210> 13
<211> 9
<212> PRT
<213> Zea mays

exemplified by determining the transit peptide sequence of maize stearoyl-ACP Δ-9 desaturase (Δ-9 desaturase) followed by producing, cloning, and expressing functional antibodies having an affinity to the same. In some cases, transit peptide sequences can be determined by comparison of the gene and amino acid sequence encoding for the complete precursor protein as described herein. Preferably, genes and nucleic acid fragments encoding for passenger proteins and the use of said genes to determine nucleic acid fragments and genes encoding transit peptides are isolated from plants. More preferred are those amino acid, gene, and nucleic acid fragments disclosed herein as SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13 SEQ ID NO:14 and SEQ ID NO:15.

Hybridoma cell lines producing monoclonal antibodies can be obtained as disclosed herein. Said cell line most preferred is 10E10 (Mab-Tp1), which was deposited in accordance with the terms of the Budapest Treaty at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, on June 18, 1998(ATTC Designation HB-12544). Monoclonal antibodies which are immunoreactive to the transit peptides disclosed herein can be produced from hybridoma cell lines. Preferably, the amino acid sequence, gene sequence and nucleic acid fragments encoding precursor proteins, transit peptides, and passenger proteins can be determined. Most preferred are those amino acid, gene and nucleic acid sequences disclosed herein as SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27 SEQ ID NO:28 SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID

tacgttacct gagcggccgc gctgggctca agttttttgt ccaccg 46

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cattctgcct ttgcggcggt tgtgatgacc ccaaacccac tc 102

<210> 21
<211> 738
<212> DNA
<213> mouse

<220>
<221> CDS
<222> (37)..(693)

<400> 21
accctcctcc tgttcnnnnn nncagntgtc ancagt gat gtt gtg atg acc cca 54
Asp Val Val Met Thr Pro
1 5

aac cca ctc tcc ctg cct gtc agt ctt gga gat caa gcc tcc atc tct 102
Asn Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser
10 15 20

tgc aga tct agt cag agc ctt tta cac agt aat gga atc acc tat tta 150
Cys Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Ile Thr Tyr Leu
25 30 35

cat tgg tac ctg cag aag cca ggc cag tct cca aag ctc ctg atc tac 198
His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr
40 45 50

aaa gtt tcc aac cga ttt tct ggg gtc cca gac agg ttc agt ggc agt 246
Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser
55 60 65 70

gga tca ggg aca gat ttc aca ctc aag atc aac aga gtg gag gct gag 294
Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Asn Arg Val Glu Ala Glu

length of transit peptides can vary, with some passenger proteins having transit peptides with as little as about 30 amino acid while other can be about 150 amino acids or longer. Nearly all transit peptide amino acid sequences nearly all start with Met-Ala at the amino terminus. The carboxy terminus can often be determined by inspection in that cleavage most often occurs at or near the motif Val-Ala-Val-Val , Val-Ala-Ala-Val or variations thereof. In many cases, transit peptides may also contain further information for organeller targeting. Depending of the source of the transit peptide, precursor proteins may display differences in import behavior, activity and efficiency. These differences are thought not to be only due solely to the transit peptide but also to passenger proteins and interaction associated therewith. Additional descriptions of transit peptide characteristics in plants and mechanisms associated therewith can be found in Pfanner et al., (1988) Eur. J. Biochem, 175:205-212; Ko and Ko, (1992) J. Biol. Chem. 267, 13910-13916; Pfanner et al., (1988) TIBS 13:165-167; Bascomb et al. (1992) Plant Microb. Biotechnol. Res. Ser. 1:142-163; and Bukau et al., (1996) Trends in Cell Biol. 6:480-486; which are incorporated herein by reference.

Transit peptides used to import proteins into the nucleus are also within the scope of this invention. Antibodies can be made against said peptides and used to lower steady state levels of the passenger proteins as disclosed herein. Nuclear transit peptides are typically comprised of short polypeptide regions and can be classified as one of three distinct types. For example, one type possesses a single short region enriched in basic amino acids. Another type is composed of two basic regions separated by a spacer. Finally, a third type possesses both hydrophobic and basic amino acids. Nuclear transit peptides in plants are further described in Silver (1991) Cell 64:489-497; Hicks et al., (1995)

```
        35                  40                  45

cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca   192
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
     50                  55                  60

gac agg ttc agt ggc agt gga tca ggg aca gat ttc aca ctc aag atc   240
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65                  70                  75                  80

aac aga gtg gag gct gag gat ctg gga gtt tat ttc tgc tct caa agt   288
Asn Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser
                85                  90                  95

aca cat gtt ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa   336
Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
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gcgcattctg cctttgcggc ggttcaactg cagcagtctg gggctgag 108

<210> 24
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<212> DNA
<213> mouse

<220>
<221> CDS
<222> (25)..(837)

<220>
<221> mat_peptide
<222> (79)..(837)

<400> 24

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                          Met Thr Ile Leu Cys Trp Leu Ala Leu
                                      -15                 -10

ctg tca act ctg act gcc gtc aac act gcg gtt gtg atg acc cca aac    99
Leu Ser Thr Leu Thr Ala Val Asn Thr Ala Val Val Met Thr Pro Asn
                -5                  -1   1                  5

cca ctc tcc ctg cct gtc agt ctt gga gat caa gcc tcc atc tct tgc   147
Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys
        10                  15                  20

aga tct agt cag agc ctt tta cac agt aat gga atc acc tat tta cat   195
Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Ile Thr Tyr Leu His
```

there is a distinct lack of amino acid and DNA homology between different transit peptides found in plants. Therefore, the use of antibodies immunologically reactive thereto in one genus, species, cultivar, or line may or may not be immunologically reactive to said transit peptides for the same passenger protein found in another genus, species, cultivar, or line. To ensure function, transit peptides from which the passenger protein of interest is derived are typically sequenced from a variety of species, cultivars, or lines to see if common amino acid motifs can be determined. If so, these common amino acid motifs can be chosen for synthetic peptide and antibody production. If said sites do not exist, individual hybridoma lines for the transit peptide of each passenger protein of interest may be required. This feature is especially important to consider in the production of hybrid crops, said crops being derived from transgenic and/or other parental lines.

Once a transit peptide is synthesized, it can be purified prior to conjugation. A variety of purification methods are available to the artisan including gel filtration, dialysis, reverse phase high performance liquid chromatography, ion exchange chromatography and the like.

After the transit peptide is synthesized and purified it may be desirable to conjugate said peptide with an appropriate carrier protein to increase the immunogenic response. A carrier protein should be a good immunogen and have a sufficient number of amino acid residues with reactive side chains for coupling to the synthetic peptide. Keyhole Limpet haemocyanin (KLH) is commonly used because of its proven efficacy. Other proteins useful as carrier molecules include thyroglobulin, bovine serum albumin, tetanus toxin, and the like.

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<211> 339
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<220>
<221> CDS
<222> (1)..(339)

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1 5 10 15

gat caa gcc tcc atc tct tgc aga tct agt cag agc ctt tta cac agt 96
Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

aat gga atc acc tat tta cat tgg tac ctg cag aag cca ggc cag tct 144
Asn Gly Ile Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca 192
Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

gac agg ttc agt ggc agt gga tca ggg aca gat ttc aca ctc aag atc 240
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

agc aga gtg gag gct gag gat ctg gga gtt tat ttc tgc tct caa agt 288
Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95

aca cat gtt ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa 336
Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

ggc 339
Gly

<210> 26
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<220>
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agtc 64

<210> 27
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<213> Artificial Sequence

antigen are typically continued until the immunological response reaches a plateau of maximal response.

After immunization is complete, immune lymphocytes from the immunized animal are typically fused to myeloma cells to generate hybridoma cell lines, which are immortal and produce monoclonal antibodies. Lymphocytes are generally taken from either lymph node tissue or spleen tissue and are fused to plasmocytoma cells, which are specialized myeloma cells available to the artisan from the American Type Culture Collection, Manassas, VA. In a typical fusion, a suspension of lymphocyte cells is added to the myeloma cells in the presence of a fusogen such as polyethylene glycol (PEG). However, other methods of cell transformation are possible including transformation of myeloma cells with viruses such as Epstein-Barr virus and the like.

After fusion, cells are harvested, diluted, and cultured for a week or more in separate wells containing the appropriate selective agent such as hypoxanthine, aminopterin, and thymidine (HAT) which is capable of extinguishing non-fused cells. Cultured supernatants from said wells can be screened to determine hybridoma growth as well as determine the selectivity and affinity of a given antibody to the antigen. The cells may be screened for the presence of antibodies capable of recognizing selected antigenic determinants by using methods available to the skilled artisan such as solid phase radioimmunoassay, enzyme-linked immunosorbent assays, western analysis, and the like. The cells so selected can then be placed in individual wells as single cell colonies by limiting dilution. Feeder cells (e.g. thymocytes, peritoneal exudate cells, and the like) may be added as necessary. After growing, supernatants therefrom can be characterized for antibody production by rescreening as described herein.

```
gta gac aaa tcc tcc agc aca gcc tac atg gag ctc cgc agc ctg aca   339
Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr
            75                  80                  85

tct gaa gac tct gcc gtc tat tac tat aca aga tgg ttt gag gac tgg   387
Ser Glu Asp Ser Ala Val Tyr Tyr Tyr Thr Arg Trp Phe Glu Asp Trp
        90                  95                  100

ggc caa ggg act ctg gtc act gtc tct gca gag ggt aaa tcc tca gga   435
Gly Gln Gly Thr Leu Val Thr Val Ser Ala Glu Gly Lys Ser Ser Gly
    105                 110                 115

tct ggc tcc gaa tcc aaa ccc ggg                                   459
Ser Gly Ser Glu Ser Lys Pro Gly
120                 125
```

<210> 30
<211> 438
<212> DNA
<213> mouse

<220>
<221> CDS
<222> (1)..(408)

<400> 30

```
gag ggt aaa tcc tca gga tct ggc tcc gaa tcc aaa ccc ggg gat gtt    48
Glu Gly Lys Ser Ser Gly Ser Gly Ser Glu Ser Lys Pro Gly Asp Val
  1               5                  10                  15

gtg atg acc cca aac cca ctc tcc ctg cct gtc agt ctt gga gat caa    96
Val Met Thr Pro Asn Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln
            20                  25                  30

gcc tcc atc tct tgc aga tct agt cag agc ctt tta cac agt aat gga   144
Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly
        35                  40                  45

atc acc tat tta cat tgg tac ctg cag aag cca ggc cag tct cca aag   192
Ile Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys
    50                  55                  60

ctc ctg atc tac aaa gtt tcc aac cga ttt tct ggg gtc cca gac agg   240
Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg
65                  70                  75                  80

ttc agt ggc agt gga tca ggg aca gat ttc aca ctc aag atc agc aga   288
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg
                85                  90                  95

gtg gag gct gag gat ctg gga gtt tat ttc tgc tct caa agt aca cat   336
Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser Thr His
            100                 105                 110

gtt ccg tac acg ttc gga ggg ggg acc aag ctg gaa ata aaa gaa gaa   384
Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Glu Glu
        115                 120                 125

aaa ctc atc tca gaa gag gat ctg aattagtaag gggccgcctt gacctagtgc  438
Lys Leu Ile Ser Glu Glu Asp Leu
    130
```

The determination of amino acid sequence allows the antibody or derivatives thereof to be synthesized using a variety of peptide synthesis methods available to the artisan including solid phase t-butyloxycarbonyl (t-BOC), solid phase 9-fluorenylmethyloxycarbonyl (t-FMOC) methods, as well as methods developed by Geysen et al., (1987, J. Immunnol. Methods, 102:259-274); Houghton et al., (1985, Proc. Natl. Acad. Sci. USA, 82:5131-5135); and the like.

In a preferred embodiment, antibodies can be prepared in vitro from chromosomal DNA, cDNA or synthetic oligonucleotides using molecular biology techniques and heterologous expression systems such as *E. coli*, yeast, Baculoviruses, and the like. Cells or other source material containing DNA or RNA coding for the desired antibody may be isolated. In some instances, it may be desirable to isolate the genomic DNA or mRNA (to generate cDNA) from such source material as described in Goldfein et al., (1987, J. Immunology, 138:940-944). When significant levels of nucleic acids are not available, one can use polymerase chain reactions (PCR) to isolate nucleic acid fragments encoding the antibody of choice, as disclosed herein.

If the amino acid sequence has been previously determined using methods disclosed herein or others available to the skilled artisan, the genetic code can be used to reverse translate said amino acid sequence into a DNA sequence. A series of oligonucleotides ranging from about 20 to about 50 bases or more can then be synthesized in order to provide a series of overlapping fragments. The synthesized oligonucleotides can be annealed and ligated to create the gene encoding said antibody.

The complete genomic sequence of a particular antibody may be obtained by screening of a genomic or cosmid library with a probe. Probes can be considerably

agc ctt tta cac agt aat gga atc acc tat tta cat tgg tac ctg cag 576
Ser Leu Leu His Ser Asn Gly Ile Thr Tyr Leu His Trp Tyr Leu Gln
155 160 165 170

aag cca ggc cag tct cca aag ctc ctg atc tac aaa gtt tcc aac cga 624
Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg
175 180 185

ttt tct ggg gtc cca gac agg ttc agc ggc agt gga tca ggg aca gat 672
Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
190 195 200

ttc aca ctc aag atc agc aga gtg gag gct gag gat ctg gga gtt tat 720
Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr
205 210 215

ttc tgc tct caa agt aca cat gtt ccg tac acg ttc gga ggg ggg acc 768
Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly Gly Gly Thr
220 225 230

aag ctg gaa ata aaa gaa gaa aaa ctt ggg tca gaa gag gat ctg 813
Lys Leu Glu Ile Lys Glu Glu Lys Leu Gly Ser Glu Glu Asp Leu
235 240 245

aattagtaag cggccgc 830

<210> 32
<211> 269
<212> PRT
<213> mouse

<400> 32
Met Val Ser Ala Ile Val Leu Tyr Val Leu Leu Ala Ala Ala Ala His
-20 -15 -10 -5

Ser Ala Phe Ala Ala Val Gln Leu Gln Glu Ser Gly Ala Glu Leu Val
-1 1 5 10

Arg Pro Gly Ala Ser Val Thr Leu Ser Cys Lys Ala Ser Gly Tyr Thr
15 20 25

Phe Thr Asp Tyr Glu Ile His Trp Val Arg Gln Thr Pro Val His Gly
30 35 40

Leu Glu Trp Ile Gly Ala Ile Asp Pro Glu Thr Gly Gly Thr Ala Tyr
45 50 55 60

Asn Gln Lys Phe Lys Asp Lys Ala Ile Val Thr Val Asp Lys Ser Ser
65 70 75

Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala
80 85 90

Val Tyr Tyr Tyr Thr Arg Trp Phe Glu Asp Trp Gly Gln Gly Thr Leu
95 100 105

Val Thr Val Ser Ala Glu Gly Lys Ser Ser Gly Ser Gly Ser Glu Ser
110 115 120

Lys Pro Gly Asp Val Val Met Thr Pro Asn Pro Leu Ser Leu Pro Val

the variable region may be altered yet the antibody may retain a functional binding site with the capacity to bind to the same antigen or epitope. Accordingly, such variations and derivations are considered to be within the scope of the present invention. Antibodies so engineered may be experimentally determined using cross-blocking techniques wherein binding of one antibody prevents binding of a second antibody to the same epitope through either local or distant steric hindrance.

Modifications contemplated by the present invention include the addition, deletion, or conservative substitution of a limited number of various amino acids. Generally, an amino acid sequence is effectively homologous to a second amino acid sequence if at least about 70 percent, preferably at least about 80 percent, and most preferably, at least about 90 percent of the amino acid sequence of the variable portions of the two antibodies in question are homologous. Conservative substitutions are those exemplified such as glutamic acid and aspartic acid; valine, leucine, and isoleucine; asparagine and glutamine; threonine and serine; glycine and alanine; phenylalanine, tyrosine, and tryptophan; and lysine and arginine. Accordingly, such variations and derivations are considered to be within the scope of the present invention.

Binding affinities between the original and homologous antibody to the antigen of choice can also be used to determine "effective homology" Effectively homologous antibodies would be expected to have antibody:antigen binding constants that are at least within about 3 orders of magnitude compared to the original antibody, and more preferably binding constants that are within about 2 orders of magnitude relative to the original antibody, with binding constants essentially the same as the original antibody being most preferred.

<210> 36
<211> 70
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 36
gcccttggtg ccctcgccgc tgcccggctt gccgctgccg ctggtgctgc cttttatttc 60

cagcttggtc 70

<210> 37
<211> 74
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 37
ggcagcacca gcggcagcgg caagccgcgc agccgccgagg gcagcaccaa gggccaggtt 60

caactgcagc agtc 74

<210> 38
<211> 72
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 38
gcactaggtc aagcggccgc atgatgatga tgatgatgag aaccccgcat tgcagagaca 60

gtgaccagag tc 72

<210> 39
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:primer

<400> 39
aaaatttgcg gccgcctaat gatgatgatg atgatgagaa c 41

<210> 40
<211> 462
<212> DNA
<213> mouse

<220>

associated with a gene transit peptide are lower than that observed in the non-transformed plant under similar conditions.

The scope of the invention as disclosed herein relates to decreased steady state levels of passenger proteins being targeted to organelles. A wide variety of modifications may be made in numerous types of plants. Preferred monocotyledonous plants, for example, include rice, corn, wheat, barley, oats, rye and sorghum. Examples of preferred dicotyledonous plants included canola, pea, soybean, sunflower, tobacco, cotton, sugar beet, petunia, tomato, broccoli, lettuce, apple, plum, orange, and lemon.

Modification to plants using the invention as disclosed herein may include varying the steady state protein levels involved in fatty acid distribution of a fatty acid source, such as rapeseed, Cuphea, corn, soybean, canola or any oilseed crop of interest. Antibodies can also be made against the transit peptide of passenger proteins such as Δ-9 desaturase, palmitoyl-ACP thioesterase, β-ketoacyl-ACP synthase, oleyl-ACP thioesterase, and the like. Antibodies can also be made against the transit peptide of proteins such as chlorophyll a/b binding proteins as a way to reduce chlorophyll content. Other organeller localized proteins having transit peptides, of which antibodies can be made using said transit peptide as an antigen as disclosed herein, include: NADPH+ dependent glyceraldehyde-3-phosphate dehydrogenase, early light inducible protein, clip protease regulatory protease, pyruvate orthophosphate dikinase, chlorophyll a/b binding protein, triose phosphate-3-phosphoglycerate phosphate translocator, 5-enol pyruval shikimate-3-phosphate synthase, dihydrofolate reductase, thymidylate synthase, acetyl-coenzyme A carboxylase, Cu/Zn superoxide dismutase, cysteine synthase, rubisco

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  1               5                  10                  15

aag ggc cat gtt caa ctg cag cag tct ggg gct gag ctg gtg agg cct   96
Lys Gly His Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro
            20                  25                  30

ggg gct tca gtg acg ctg tcc tgc aag gct tcg ggc tac aca ttt act   144
Gly Ala Ser Val Thr Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
        35                  40                  45

gac tat gaa ata cac tgg gtg aag cag aca cct gtg cat ggc ctg gaa   192
Asp Tyr Glu Ile His Trp Val Lys Gln Thr Pro Val His Gly Leu Glu
    50                  55                  60

tgg att gga gct att gat cct gaa act ggt ggt act gcc tac aat cag   240
Trp Ile Gly Ala Ile Asp Pro Glu Thr Gly Gly Thr Ala Tyr Asn Gln
65                  70                  75                  80

aag ttc aag gac aag gcc ata ctg act gca gac aaa tcc tcc agc aca   288
Lys Phe Lys Asp Lys Ala Ile Val Thr Val Asp Lys Ser Ser Ser Thr
                85                  90                  95

gcc tac atg gag ctc cgc agc ctg aca tct gaa gac tct gcc gtc tat   336
Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr
            100                 105                 110

tac tat aca aga tgg ttt gag gac tgg ggc caa ggg act ctg gtc act   384
Tyr Tyr Thr Arg Trp Phe Glu Asp Trp Gly Gln Gly Thr Leu Val Thr
        115                 120                 125

gtc tct gca atg cgg ggt tct cat cat cat cat cat cat gcggccgctt     433
Val Ser Ala Met Arg Gly Ser His His His His His His
    130                 135                 140

gacctagtgc                                                        443


<210> 42
<211> 851
<212> DNA
<213> mouse

<220>
<221> CDS
<222> (19)..(831)

<220>
<221> mat_peptide
<222> (73)..(831)

<400> 42
gcactaggtc aatctaga atg act atc ctt tgc tgg cta gcc ctt ctg tca   51
                  Met Thr Ile Leu Cys Trp Leu Ala Leu Leu Ser
                      -15                 -10

act ctg act gcc gtc aac gct gag gtt ctg atg acc cca aac cca ctc   99
Thr Leu Thr Ala Val Asn Ala Ala Val Val Met Thr Pro Asn Pro Leu
        -5                  -1   1                  5

tcc ctg cct gtc agt ctt gga gat caa gcc tcc atc tct tgc aga tct   147
Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser
```

To obtain high expression of heterologous genes in plants it may be preferred to reengineer said genes so that they are more efficiently expressed in the cytoplasm of plant cells. Maize is one such plant where it may be preferred to reengineer the heterologous gene(s) prior to transformation to increase the expression level thereof in said plant. Therefore, an additional step in the design of genes encoding antibodies for plant expression may involve reengineering of a heterologous gene for optimal expression.

One reason for the reengineering an antibody gene for expression in maize is to ensure the optimal G+C content of the native gene as well as eliminate any sequences mimicking or duplicating plant gene control sequences. The presence of some A+T-rich sequences within the DNA of gene(s) introduced into plants (e.g., TATA box regions normally found in gene promoters) may result in aberrant transcription of the gene(s). On the other hand, the presence of other regulatory sequences residing in the transcribed mRNA (e.g., polyadenylation signal sequences (AAUAAA), or sequences complementary to small nuclear RNAs involved in pre-mRNA splicing) may lead to RNA instability. Therefore, one aspect in the design of genes encoding an antibody gene for maize expression, more preferably referred to as plant optimized gene(s), is to generate a DNA sequence having a higher G+C content, and preferably one close to that of maize genes coding for metabolic enzymes. Another goal in the design of the plant optimized gene(s) is to generate a DNA sequence in which the sequence modifications do not hinder translation.

The table below (Table 1) illustrates how high the G+C content is in maize. For the data in Table 1, coding regions of the genes were extracted from GenBank (Release 71) entries, and base compositions were calculated using

<210> 43
<211> 867
<212> DNA
<213> mouse

<220>
<221> CDS
<222> (31)..(843)

<220>
<221> mat_peptide
<222> (85)..(843)

<400> 43
aatttaaacg gatccgggt cattctaga atg act atc ctt tgc tgg cta gcc 54
Met Thr Ile Leu Cys Trp Leu Ala
-15

ctt ctg tca act ctg act gcc gtc aac gct gcg gtt gtg atg acc cca 102
Leu Leu Ser Thr Leu Thr Ala Val Asn Ala Ala Val Val Met Thr Pro
-10 -5 -1 1 5

aac cca ctc tcc ctg cct gtc agt ctt gga gat caa gcc tcc atc tct 150
Asn Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser
10 15 20

tgc aga tct agt cag agc ctt tta cac agt aat gga atc acc tat tta 198
Cys Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Ile Thr Tyr Leu
25 30 35

cat tgg tac ctg cag aag cca ggc cag tct cca aag ctc ctg atc tac 246
His Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr
40 45 50

aaa gtt tcc aac cga ttt tct ggg gtc cca gac agg ttc agt ggc agt 294
Lys Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser
55 60 65 70

gga tca ggg aca gat ttc aca ctc aag atc agc aga gtg gag gct gag 342
Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu
75 80 85

gat ctg gga gtt tat ttc tgc tct caa agt aca cat gtt ccg tac acg 390
Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr
90 95 100

ttc gga ggg ggg acc aag ctg gaa ata aaa ggc agc acc agc ggc agc 438
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly Ser Thr Ser Gly Ser
105 110 115

ggc aag ccg ggc agc ggc gag ggc agc acc aag ggc cat gtt caa ctg 486
Gly Lys Pro Gly Ser Gly Glu Gly Ser Thr Lys Gly His Val Gln Leu
120 125 130

cag cag tct ggg gct gag ctg gtg agg cct ggg gct tca gtg acg ctg 534
Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val Thr Leu
135 140 145 150

tcc tgc aag gct tcg ggc tac aca ttt act gac tat gaa ata cac tgg 582

resulting nucleic acid sequence codes for exactly the same protein as the native gene wanting to be heterologously expressed. The new

Table 1. Compilation of G+C contents of protein coding regions of maize genes.

Protein Class[a]	Range %G+C	Mean %G+C[b]
Metabolic Enzymes (76)	44.4-75.3	59.0 (± 8.0)
Structural Proteins (18)	48.6-70.5	63.6 (± 6.7)
Regulatory Proteins (5)	57.2-68.9	62.0 (± 4.9)
Uncharacterized Proteins (9)	41.5-70.3	64.3 (± 7.2)
All Proteins (108)	**44.4-75.3**	**60.8 (± 5.2)**

[a]Number of genes in class given in parentheses.

[b]Standard deviations given in parentheses.

[c]Combined groups mean ignored in mean calculation.

DNA sequences are designed using codon bias information so that they correspond to the most preferred codons of the desired plant. The new sequences are then analyzed for restriction enzyme sites that might have been created by the modifications. The identified sites are further modified by replacing the codons with second or third choice with preferred codons. Other sites in the sequence which could affect transcription or translation of the gene of interest are the exon:intron 5′ or 3′ junctions, poly A addition signals, or RNA polymerase termination signals. The sequence is further analyzed and modified to reduce the frequency of TA or GC doublets. In addition to the doublets, G or C sequence blocks that have more than about four residues that are the same can affect transcription of the sequence. Therefore, these blocks are also modified by replacing the codons of first or second choice, etc. with the next preferred codon of choice.

tttaaaggcc agagaggccc taatgatgat gatgatgatg agaaccccgc attg 54

<210> 47
<211> 882
<212> DNA
<213> mouse

<220>
<221> CDS
<222> (25)..(837)

<220>
<221> mat_peptide
<222> (79)..(837)

<400> 47
ctgcagggta cggccatatt ggcc atg act acc ctt tgc tgg ctg gcc ctt 51
Met Thr Ile Leu Cys Trp Leu Ala Leu
-15 -10

ctg tca act ctg act gcc gtc aac act gcg gtt gtg atg acc cca aac 99
Leu Ser Thr Leu Thr Ala Val Asn Thr Ala Val Val Met Thr Pro Asn
-5 -1 1 5

cca ctc tcc ctg cct gtc agt ctt gga gat caa gcc tcc atc tct tgc 147
Pro Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys
10 15 20

aga tct agt cag agc ctt tta cac agt aat gga atc acc tat tta cat 195
Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Ile Thr Tyr Leu His
25 30 35

tgg tac ctg cag aag cca ggc cag tct cca aag ctc ctg atc tac aaa 243
Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys
40 45 50 55

gtt tcc aac cga ttt tct ggg gtc cca gac agg ttc agt ggc agt gga 291
Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly
60 65 70

tca ggg aca gat ttc aca ctc aag atc agc aga gtg gag gct gag gat 339
Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp
75 80 85

ctg gga gtt tat ttc tgc tct caa agt aca cat gtt ccg tac acg ttc 387
Leu Gly Val Tyr Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe
90 95 100

gga ggg ggg acc aag ctg gaa ata aaa ggc agc acc agc ggc agc ggc 435
Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly Ser Thr Ser Gly Ser Gly
105 110 115

aag ccg ggc agc ggc gag ggc agc acc aag ggc cat gtt caa ctg cag 483
Lys Pro Gly Ser Gly Glu Gly Ser Thr Lys Gly His Val Gln Leu Gln
120 125 130 135

cag tct ggg gct gag ctg gtg agg cct ggg gct tca gtg acg ctg tcc 531
Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val Thr Leu Ser
140 145 150

transgenic tobacco. Other strategies for increasing expression of antibody genes have been explored by Owen et al., (1992, Chem. Ind., 11:406-408); Ma (1995, ACS Symp. Ser., 604:56-59); Schouten et al., (1997 FEBS Lett. 415:235-241); Conrad and Fiedler, (1994, Plant Mol. Biol. 26:1023-1030); Fiedler et al., (1997, Immunotechnology 3:205-216); van Engelen et al., (1994, Plant Mol. Biol. 26:1701-1710); Hiatt et al., (1989, Nature 342: 76-78); Firek et al., (1993, Plant Mol. Biol. 23:861-870); Ma et al., (1994, Eur. J. Immunol. 24:131-138), which are incorporated herein by reference.

In another aspect of the invention, genes encoding the antibodies are expressed from transcriptional units inserted into the plant genome. Preferably, said transcriptional units are recombinant vectors capable of stable integration into the plant genome and selection of transformed plant lines expressing mRNA encoding for said antibodies are expressed either by constitutive or inducible promoters in the plant cell. Once expressed, mRNA is translated into proteins, thereby incorporating amino acids of interest. Genes encoding antibodies expressed in plant cells can be under the control of a constitutive promoter, a tissue-specific promoter, an inducible promoter, and the like. Several techniques exist for introducing foreign recombinant vectors into plant cells, and for obtaining plants that stably maintain and express the introduced gene. Such techniques include acceleration of genetic material coated onto microparticles directly into cells (U.S. Patents 4,945,050 to Cornell and 5,141,131 to DowElanco, now Dow AgroSciences, LLC). In addition, plants may be transformed using *Agrobacterium* technology, see U.S. Patent 5,177,010 to University of Toledo, 5,104,310 to Texas A&M, European Patent Application 0131624B1, European Patent Applications 120516, 159418B1 and 176,112 to Schilperoot, U.S. Patents 5,149,645, 5,469,976,

```
ggg aca gat ttc aca ctc aag atc agc aga gtg gag gct gag gat ctg   291
Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu
 75                  80                  85

gga gtt tat ttc tgc tct caa agt aca cat gtt ccg tac acg ttc gga   339
Gly Val Tyr Phe Cys Ser Gln Ser Thr His Val Pro Tyr Thr Phe Gly
 90                  95                 100                 105

ggg ggg acc aag ctg gaa ata aaa ggc agt acc agc ggc agc ggc aag   387
Gly Gly Thr Lys Leu Glu Ile Lys Gly Ser Thr Ser Gly Ser Gly Lys
                110                 115                 120

ccg ggc agc ggc gag ggc agc acc aag ggt cat gtt caa ctg cag cag   435
Pro Gly Ser Gly Glu Gly Ser Thr Lys Gly His Val Gln Leu Gln Gln
            125                 130                 135

tct ggg gct gag ctg gtg agg cct ggg gct tca gtg acg ctg tcc tgc   483
Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Ser Val Thr Leu Ser Cys
        140                 145                 150

aag gct tcg ggc tac aca ttt act gac tat gaa ata cac tgg gtg agg   531
Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Glu Ile His Trp Val Arg
    155                 160                 165

cag aca cct gtg cat ggc ctg gaa tgg att gga gct att gat cct gaa   579
Gln Thr Pro Val His Gly Leu Glu Trp Ile Gly Ala Ile Asp Pro Glu
170                 175                 180                 185

act ggt ggt act gcc tac aat cag aag ttc aag gac aag gcc ata gtg   627
Thr Gly Gly Thr Ala Tyr Asn Gln Lys Phe Lys Asp Lys Ala Ile Val
                190                 195                 200

act gta gac aaa tcc tct agc aca gcc tac atg gag ctc cgc agc ctg   675
Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu
            205                 210                 215

aca tct gaa gac tct gcc gtc tat tac tgt aca aga tgg ttt gag gac   723
Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Thr Arg Trp Phe Glu Asp
        220                 225                 230

tgg ggc caa ggg act ctg gtc act gtc tct gca atg cgg ggt tct cat   771
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Met Arg Gly Ser His
    235                 240                 245

cat cat cat cat cat tagggcctct ctggccgatc ccccgaattt ccccgatcgt   826
His His His His His
250

tcaaacattt ggcaataaag                                             846
```

<210> 49
<211> 738
<212> DNA
<213> mouse

<220>
<221> CDS
<222> (19)..(687)
<223> mature peptide is coded by nucleotides 49 to 687

rhizogenes as a mode for transformation, although T-DNA borders may find use with other modes of transformation.

Where Agrobacterium is used for plant cell transformation, a vector may be used which may be introduced into the host for homologous recombination with T-DNA or the Ti or Ri plasmid present in the host. Introduction of the vector may be performed via electroporation, tri-parental mating and other techniques for transforming gram-negative bacteria which are known to those skilled in the art. The manner of vector transformation into the Agrobacterium host is not critical to this invention. The Ti or Ri plasmid containing the T-DNA for recombination may be capable or incapable of causing gall formation, and is not critical to said invention so long as the *vir* genes are present.

Table 2. Preferred amino acid codons for proteins expressed in maize.

Amino Acid	Codon*
Alanine	GCC/GCG
Cysteine	TGC/TGT
Aspartic Acid	GAC/GAT
Glutamic Acid	GAG/GAA
Phenylalanine	TTC/TTT
Glycine	GGC/GGG
Histidine	CAC/CAT
Isoleucine	ATC/ATT
Lysine	AAG/AAA
Leucine	CTG/CTC
Methionine	ATG
Asparagine	AAC/AAT
Proline	CCG/CCA
Glutamine	CAG/CAA
Arginine	AGG/CGC
Serine	AGC/TCC
Threonine	ACC/ACG

agaaccaaaa a 738

<210> 50
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Ala His Ser Ala Phe Ala Ala Val Gln Leu Gln Gln Ser Gly Ala Glu
     -5              -1   1                   5                  10

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Leu Val Arg Pro Gly Ala Ser Val Thr Leu Ser Cys Lys Ala Ser Gly
                 15                  20                  25

tac aca ttt act gac tat gaa ata cac tgg gtg agg cag aca cct gtg   192
Tyr Thr Phe Thr Asp Tyr Glu Ile His Trp Val Arg Gln Thr Pro Val
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cat ggc ctg gaa tgg att gga gct att gat cct gaa act ggt ggt act   240
His Gly Leu Glu Trp Ile Gly Ala Ile Asp Pro Glu Thr Gly Gly Thr
         45                  50                  55

gcc tac aat cag aag ttc aag gac aag gcc ata gtg act gta gac aaa   288
Ala Tyr Asn Gln Lys Phe Lys Asp Lys Ala Ile Val Thr Val Asp Lys
     60                  65                  70

tcc tcc agc aca gcc tac atg gag ctc cgc agc ctg aca tct gaa gac   336
Ser Ser Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp
 75                  80                  85                  90

tct gcc gtc tat tac tgt aca aga tgg ttt gag gac tgg ggc caa ggg   384
Ser Ala Val Tyr Tyr Cys Thr Arg Trp Phe Glu Asp Trp Gly Gln Gly
                 95                  100                 105

act ctg gtc act gtc tct gca gcc aaa aca aca ccc cca tca gtc tat   432
Thr Leu Val Thr Val Ser Ala Ala Lys Thr Thr Pro Pro Ser Val Tyr
             110                 115                 120
```

express said gene in a plant cell by including in the vector a plant promoter regulatory element, as well as 3' non-translated transcriptional termination regions such as Nos and the like.

In addition to numerous technologies for transforming plants, the type of tissue which is contacted with the foreign genes may vary as well. Such tissue would include but would not be limited to embryogenic tissue, callus tissue types I, II, and III, hypocotyl, meristem, root tissue and the like. Almost all plant tissues may be transformed during cellular dedifferentiation using appropriate techniques described herein.

Another variable is the choice of a selectable marker. Preference for a particular marker is at the discretion of the artisan, but any of the following selectable markers may be used along with any other gene not listed herein which could function as a selectable marker. Such selectable markers include but are not limited to aminoglycoside phosphotransferase gene of transposon Tn5 (Aph II) which encodes resistance to the antibiotics kanamycin, neomycin and G418, as well as those genes which encode for resistance or tolerance to glyphosate; hygromycin; methotrexate; phosphinothricin (bialophos); imidazolinones, sulfonylureas and triazolopyrimidine herbicides, such as chlorsulfuron; bromoxynil, dalapon and the like.

In addition to a selectable marker, it may be desirous to use a reporter gene. In some instances a reporter gene may be used with or without a selectable marker. Reporter genes are genes which are typically not present in the recipient organism or tissue and typically encode for proteins resulting in some phenotypic change or enzymatic property. Examples of such genes are provided in K. Wising et al. (1988) Ann. Rev. Genetics, 22:421, which is incorporated herein by reference.

of pDAB439

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of pDAB439

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<211> 24
<212> PRT
<213> Zea mays

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1 5 10 15

Pro Glu Ser Leu Asp Val Arg Gly
20

optimal performance of the transformed DNA in the plant. Typical elements include but are not limited to Adh-intron 1, Adh-intron 6, the alfalfa mosaic virus coat protein leader sequence, the maize streak virus coat protein leader sequence, as well as others available to a skilled artisan.

Constitutive promoter regulatory elements may also be used thereby directing continuous gene expression in all cells types and at all times (e.g., actin, ubiquitin, CaMV 35S, and the like). Tissue specific promoter regulatory elements are responsible for gene expression in specific cell or tissue types, such as the leaves or seeds (e.g., zein, oleosin, napin, ACP, globulin, and the like) and these may also be used.

Promoter regulatory elements may also be active during a certain stage of the plants' development as well as active in plant tissues and organs. Examples of such include but are not limited to pollen-specific, embryo specific, corn silk specific, cotton fiber specific, root specific, seed endosperm, fruit-specific, specific promoter regulatory elements and the like. Under certain circumstances it may be desirable to use an inducible promoter regulatory element, which is responsible for expression of genes in response to a specific signal, such as: physical stimulus (heat shock genes); light (RUBP carboxylase); hormone (Em); metabolites; chemical; and stress. Other desirable transcription and translation elements that function in plants may be used.

The present invention also contemplates the use of transformed plants which are selfed to produce an inbred plant. The inbred plant can produce seed containing genes encoding antibodies. The inbred lines can also be crossed with other inbred lines to produce hybrids. Parts obtained from the regenerated plant, as flowers, seeds, leaves branches, fruit and the like are covered by the invention provided that said parts contain genes

The invention is illustrated in further details by the following non-limiting examples. The examples are for the purpose of illustration only and are not to be construed as limiting the scope of the present invention.

EXAMPLE 1

ISOLATION AND CLONING OF STEAROYL-ACP Δ-9 DESATURASE (Δ-9 DESATURASE) FROM MAIZE

A cDNA clone encoding maize stearoyl-ACP Δ-9 desaturase (Δ-9 desaturase) was obtained from a cDNA library derived from maize kernels of inbred CS608 (Mycogen Seeds, San Diego, CA) that had been grown in a greenhouse and hand pollinated. The cDNA library was prepared from said kernels harvested at 20 days after pollination, hereinafter 20-DAP. Upon harvest, embryos were immediately collected, frozen on dry ice, and stored at -70° C. RNA was extracted by grinding 2.5 g to a fine powder in liquid nitrogen. Afterwards, 10 mL of extraction buffer [50 mM Tris-HCl, pH 8.0, 4% para-amino salicyclic acid (Sigma Chemical Co), 1% tri-iso-propylnaphtalenesulfonic acid (Eastman Kodak Co., Rochester, NY), 10 mM DTT, and 10 mM sodium meta-bisulfite (Sigma Chemical Co., St. Louis, MO)] was added and the mixture was homogenized for 1 min using a TEKMAR TISSUMIZER (Tekmar Co., Cincinnati, OH). The homogenate was extracted with an equal volume of phenol equilibrated with 0.1 M Tris-HCl, pH 8.0. Organic and aqueous phases were separated by centrifugation at 4° C. The aqueous phase was removed and extracted with an equal volume of chloroform/octanol (24:1). The supernatant was then transferred, centrifuged, transferred again, and a one-half volume of 7.5 M ammonium acetate (pH 8.0) was added. RNA was then precipitated on ice for 30 min.

Precipitated RNA was collected by centrifugation and dissolved in 1 mL of diethylpyrocarbonate-treated water

ZAP-cDNA synthesis and cloning kit according to the manufacturers protocols (Stratagene, La Jolla, CA). The resulting library had an original titer of 3.38×10^{10} plaque forming units/mL (pfu/mL), greater than 95% recombinants and an average insert size of 1.35 kb. The cDNA library was amplified according to Sambrook et al., (Molecular Cloning, A Laboratory Manual, 2nd Ed. (1989) Cold Spring Harbor Laboratory Press) and had a titer of 6.0×10^6 pfu/mL. Total library cDNA was batch rescued and isolated as follows: 5 mL of XL1 Blue *E. coli* cells (Stratagene) at OD_{600nm} = 1.0 in 10 mM $MgSO_4$ were mixed with 8.3 µL ($5x10^8$ pfu) of amplified embryo cDNA library phage-stock, and 100 µL EXASSIST helper phage (Stratagene) and incubated at 37° C for 20 min. Twenty-five mL of TY medium [8.0 g/L tryptone, 5.0 g/L yeast extract, and 2.5 g/L NaCl, pH 7.8] were added and cells were incubated at 37° C for 3 h while shaking. Afterwards, bacterial cells were heat killed at 68° C for 15 min and the supernatant was recovered. Five hundred µL supernatant was mixed with 14.5 mL of SOLR cells (Stratagene) (OD_{600nm} = 1.5), incubated at 37° C for 15 min, added to 500 mL LB [10 g/L tryptone, 10 g/L NaCl, and 5 g/L yeast extract containing 50 µg/mL Ampicillin], and grown overnight. Afterwards, plasmid DNA was obtained by alkaline lysis/CsCl purification, according to Sambrook et al (Molecular Cloning, A Laboratory Manual, 2nd Ed. (1989) Cold Spring Harbor Laboratory Press), which is incorporated by reference, and analyzed by agarose gel electrophoresis following digestion with EcoRI/XhoI. A smear ranging from 0.5 to 3.0 kb was observed following electrophoresis.

To isolate a clone encoding maize Δ-9 desaturase, a DNA fragment was amplified using polymerase chain reaction technology, hereinafter PCR, to produce a probe which could be used to isolate a full length cDNA. A 5'

The CS608 embryo cDNA library described herein was screened using a DNA fragment which was essentially SEQ ID NO:3. This fragment was used to obtain full length clones encoding maize Δ-9 desaturase. Probe DNA was obtained by digesting the cloned PCR fragment (SEQ ID NO:3) with the appropriate restriction enzymes. This material was then run on a preparative 1% agarose gel, the band was excised and the DNA was extracted with QIAEX (Qiagen). An α^{32}P-deoxyribocytosine triphosphate (dCTP)-labeled probe was generated using QUICKPRIME Random Labeling kit (Stratagene, LaJolla, CA) according to the manufacturer instructions using 5 µL of [α^{32}P]-dCTP (3000 Ci/mmole, 10 µCi/µL, DuPont, NEN Life Science Products, Boston, MA). Afterwards, the labeling reaction was applied to a NucTrap column (Stratagene) equilibrated with TE [10 mM Tris-HCl, pH 8.0, 1 mM EDTA]. Labeled DNA was eluted with 2 volumes of TE (400 µL each). The probe was heat denatured before being added to hybridization buffer as described herein.

Methods for phage titering, plating, coring and rescuing were as described in the LAMBDA ZAP II Library (Stratagene) instruction manual. The cDNA library was plated (100,000 pfu/plate) on five 22.5 x 22.5 cm NUNC assay plates (Nunc Inc. Roskilde, Denmark). Duplicate phage lifts were taken from each plate using 0.45 µm Hybond N+ nylon membranes (Amersham, Arlington Heights, IL). Following transfer of the phage to the filters, cells and phage were denatured and DNA was fixed to the membranes by autoclaving for 8 min at 125° C followed by UV-crosslinking using a STRATALINKER UV Crosslinker (Stratagene).

Prior to hybridization, filters were extensively washed in 2x SSC [1x SSC is 150 mM NaCl, 15 mM sodium citrated, pH 7.0] 0.1% sodium dodecyl sulfate (SDS) at 65° C. Filter prehybridization was performed at 42° C for 1

agar containing Ampicillin (75 µg/mL) and grown overnight at 37° C. DNA was extracted from 4 mL liquid cultures grown overnight at 37° C in TB [1.2% tryptone, 2.4% yeast extract, 0.4% glycerol, 0.17 M KH_2PO_4, and 0.72 M K_2HPO_4] using the alkaline lysis/polyethylene glycol protocol described in the PRISM READY REACTION DYEDEOXY Terminator Cycle Sequencing Kit Protocol#401388 Rev. B (Perkin-Elmer, Applied Biosystems Division). A sequence of the full length maize Δ-9 desaturase cDNA and corresponding amino acid sequence is entered herein as SEQ ID NO:5 and SEQ ID NO:6, respectively.

EXAMPLE 2

EXPRESSION OF THE GENES ENCODING MAIZE STEAROYL-ACP Δ-9 DESATURASE (Δ-9 DESATURASE) MATURE PROTEIN AND PRECURSOR PROTEIN

The coding sequence of maize Δ9 desaturase was cloned in expression vector pET9d DN using standard molecular cloning techniques. pET9d DN is a modified version of expression vector pET9d (Novagen Inc., Madison, WI) allowing subcloning of fragments in a unique NheI site downstream from the Shine & Dalgarno sequence and translation initiation ATG codon. To facilitate subcloning the cDNA clone (SEQ ID NO:5) was amplified with primers having the sequence entered herein as SEQ ID NO:7 and SEQ ID NO:8. Following amplification, the PCR product was digested with an excess of NheI, purified through agarose gel and directly ligated into vector pET9d DN which had been cut with NheI and dephosphorylated with calf intestinal phosphatase. The ligation was transformed into *E. coli* DH5α cells and recombinant plasmids containing inserts were selected by miniprep screening and digestion with NheI. One clone with the appropriate sized insert in the correct orientation relative to the T7 promoter was identified by

soluble and pellet fractions on 10-15% gradient SDS polyacrylamide Phast Gels (Pharmacia LKB Biotechnology, Piscataway, NJ). Protein bands were visualized by staining with Coomassie Brilliant Blue in the Phast Gel apparatus, and destained overnight by shaking in a small volume of 10% glycerol, 10% acetic acid. Molecular size of bands was determined by comparison to Bio-Rad Low Molecular Weight markers (Bio-Rad Laboratories, Hercules, CA). Western blot analysis was performed using antibodies produced as described herein specific to the mature portion of maize Δ9-desaturase. Expression of plasmid pDAB432D in *E.coli* resulted in the production of a 42 kDa protein in the cell pellet, indicating that the precursor protein produced in *E. coli* was insoluble.

In production of mature Δ-9 desaturase protein, the portion of the cDNA clone encoding the mature desaturase protein was subcloned into pETd DΔN. The amino terminus of the mature maize Δ-9 desaturase protein determined as described herein. In order to express the mature protein, DNA corresponding to SEQ ID NO:11 was cloned into the expression vector pET9dΔN which is a modified version of pET9d (Novagen, Inc.). The vector pET9dΔN allowed subcloning of fragments in a unique NheI site downstream from the Shine and Dalgarno sequence and translation initiation ATG codon.

To facilitate subcloning, DNA corresponding to SEQ ID NO:5 was amplified with primers entered herein as SEQ ID NO:9 and SEQ ID NO:10. The DNA product had the sequence entered herein as SEQ ID NO:11 and encoded a protein having the amino acid sequence entered herein as SEQ ID NO:12. Following amplification, the PCR product was digested with an excess of NheI, gel purified through agarose gel and directly ligated into vector pET9dΔN which had been cut with NheI and dephosphorylated with calf intestinal phosphatase. The ligation was

aldolase (158.0 kDa); catalase (232.0 kDa); ferritin (440.0 kDa) and blue dextran (2,000 kDa).

The putative protein of interest was subjected to SDS-PAGE, blotted onto ProBlott membrane (Amersham), visualized with 0.02% amido black, excised, and sent to Harvard Microchem, (Boston, MA) for amino-terminal sequence analysis thus yielding the sequence entered herein as SEQ ID NO:13. Spectrophotometric analysis of the diiron-oxo component associated with the expressed protein (Fox et al., 1993, Proc. Natl. Acad. Sci. USA 90:2486-2490), as well as identification using a stain specific for nonheme iron (Leong et al., 1992, Anal. Biochem. 207:317-320) were also used to confirmed that the protein was mature Δ-9 desaturase.

The *E. coli* produced mature Δ-9 desaturase, as determined by amino terminal sequencing, was gel purified via SDS-PAGE and sent in the gel matrix to Berkeley Antibody Co. (Richmond, CA) for production of polyclonal antiserum in rabbits. Inoculations of antigen were initiated with 200 µg protein followed by three boosting injections of 100 µg each at three week intervals. Evaluations of antibody titers against mature Δ-9 desaturase were performed via western analysis using the ECL detection system (Amersham, Inc.; Arlington Heights, IL).

Polyclonal antiserum produced above were used for western analysis as follows: purified or partially purified proteins were subjected to SDS-PAGE analysis on a 10-20% polyacrylamide gel (Integrated Separation Systems, Natick, MA) using the method described by Laemmli (1970, Nature 277:680). Proteins were then transferred to nitrocellulose paper using a Pharmacia Semi-Dry Blotter and the nonspecific binding sites were blocked with BLOTTO (5% dry milk in phosphate buffered saline [PBS]/0.05% Tween-20). The membrane was incubated in the polyclonal media (polyclonal antibody diluted

bromide-activated Sepharose 4-B beads was performed essentially as described by Pharmacia, Inc., in the package insert. After linkage and blocking of the remaining sites with glycine, the ACP-sepharose material was packed into a HR 5/5 column (Pharmacia, Inc.) and equilibrated in 25 mM sodium phosphate buffer (pH 7.0). Mono-S fractions identified as having Δ-9 desaturase activity were then loaded onto the ACP-Sepharose column as described previously (McKeon and Stumpf, 1982, J. Biol. Chem. 254:7116-7122; Thompson et al., 1991, J. Biol. Chem. 257:12141-12147). After extensive column washing, ACP-binding proteins were eluted using 1M NaCl.

Fractions containing Δ-9 desaturase activity obtained from the ACP-sepharose column were adjusted to 0.4 M ammonium sulfate (25 mM sodium phosphate, pH 7.0) and loaded onto a Pharmacia Phenyl Superose column (HR 10/10). Proteins were eluted by running a gradient (0.4 - 0.0 M ammonium sulfate) at 2 mL/min for 1 hr. Δ-9 desaturase activity typically eluted between 60- and 30 mM ammonium sulfate as determined by enzymatic and western analysis. Proteins were dialyzed extensively and concentrated using a Centricon-10 concentrator (Amicon, Inc., Beverly, MA). Samples were then blotted to PVDF membrane as described herein and sent to Harvard Microchem for amino terminal analysis. The amino terminal sequence is entered herein as SEQ ID NO:14.

Production of substrate for Δ-9 desaturase activity assays was performed as follows. Cells harboring a plasmid encoding acyl-ACP synthetase (Jackowski et al., 1994, J. Biol. Chem. 269:2921-2928) were grown, heat shock induced, and the acyl-ACP synthetase protein produced therein was partially purified essentially as described by Rock and Cronan, (1979, J. Biol. Chem. 254:7116-7122). [9,10 (n)- ^{3}H] stearic acid (specific activity = 49 Ci/mmol) was custom synthesized by Amersham

As described herein, the maize Δ-9 desaturase gene was cloned and the amino acid sequence of the putative transit peptide (Δ-9TP) was determined and entered herein as SEQ ID NO:15. This sequence information was sent to Bio-Synthesis, Inc., (Lewisville, TX) for peptide synthesis. Once received, the peptide was dissolved in sterile water and aliquots thereof were analyzed by gel electrophoresis on a 4-27% gradient gel (Integrated Separation Science, Inc., Natick, MA). By comparing to the molecular weight markers, the synthetic Δ-9TP was determined to be about 4.0 kDa. The synthetic Δ-9TP was then conjugated to Keyhole Limpet hemacyanin (KLH) and injected into rabbits for polyclonal antibody production by BioSynthesis, Inc. Serum was taken at 10 weeks and 13 weeks after the initial injection. Polyclonal antibodies derived from this production were so named Pab-TP.

Immunospecificity of Pab-TP to the Δ-9TP peptide was determined by Western blot analysis using *E. coli* produced precursor (pDAB432D) and mature Δ-9 desaturase protein (pDAB428), as well as the synthetically made Δ-9TP polypeptide. Proteins were loaded onto a SDS-PAGE gel as described herein and were then blotted to PVDF membrane for western analysis as previously described. In testing Pab-TP antibodies, it was observed that said antibodies were immunologically reactive to both the precursor Δ-9 desaturase and the Δ-9TP polypeptide but did not recognize the mature protein. These data indicated that Pab-TP antibodies were specific for the transit peptide portion only of maize Δ-9 desaturase.

EXAMPLE 5
PRODUCTION AND SCREENING OF HYBRIDOMA CELL LINES

mixture was incubated at 37° C for 20 min. The medium was removed by centrifugation. Cell pellets were then resuspended in 200 mL of selection medium containing: complete medium [500 mL RPMI 1640, 50 mL fetal bovine serum (Sigma Chemical Co.), 6 mL 200 mM L-glutamine (Cellgro™), 6 mL 100 mM sodium pyruvate (Cellgro™), 5000 units penicillin-streptomycin (Cellgro™)], 10% Origen Hybridoma Cloning Factor (HCF; Origen, Fisher Scientific), and 1x HAT solution (hypoxanthine, aminopterin, and thymidine (Cellgro™). Cells were incubated for 1 h at 37° C and 200 µL thereof were distributed into each well of 96-well plates. After 10 to 14 days, media from each well were screened for antibody-producing hybridomas.

ELISA techniques were used to screen for Δ-9TP specific monoclonal antibody (Mab) producing hybridoma cell lines. The assay involved first applying the antigen (10-50 ng of synthetic Δ-9TP or precursor protein produced from pDAB432D) in 0.1 M sodium carbonate into wells of microtiter plates (Dynatech Laboratories, Chantilly, Virginia) and incubating overnight at 4° C. Afterwards, the excess antigen solution was discarded and the wells were blocked with 1% BSA (bovine serum albumin) in PBS (phosphate buffer saline, Sigma) at 37° C for at least 1 h. The blocking solution was discarded, and 100 µL of hybridoma cell medium was added to each well. Plates were incubated at 37° C for at least 1 h, after which they were washed 3 times with wash solution (0.025% Tween 20 in PBS). A 100 µL volume of a 1:1000 dilution of alkaline phosphatase conjugated anti-IgG + IgM antibodies (Kirkegaard & Perry, Gaithersburg, MD) in 1% BSA in PBS was added to each well. The plates were incubated at 37° C for 1 h and washed 3 times with the wash solution. Finally, the phosphatase substrate p-NPP

callus tissue, Type II corn callus tissue, maize seed extract, *E.coli* produced precursor protein (pDAB432D), and purified mature Δ-9 desaturase protein. Duplicate blots were made and each blot was incubated with MAb-TP1, or MAb-Δ9M. The result showed that MAb-TP1 could only recognize Δ-9 desaturase precursor protein and did not recognize mature Δ9-desaturase or only other proteins in callus tissues or seeds. These data indicated that the Mabs generated against Δ-9TP were specific thereto.

EXAMPLE 6

CLONING OF GENES FROM MONOCLONAL CELL LINES

Hybridoma cell lines of interest were grown in two 150 mL flasks containing 60 mL of hybridoma medium. Seven days after inoculation, about 1 x 10^8 cells were harvested into 50 mL centrifuge tubes and pelleted by centrifugation at 800 rpm using a table-top centrifuge. The medium was discarded and the cells were washed twice with sterile PBS. Tubes containing washed cells were placed on dry ice for 15 min prior to mRNA isolation.

PolyA+ mRNA was isolated using a Fast Track mRNA Isolation Kit (Invitrogen, Carlsbad, CA). Hybridoma cells were resuspended in 15 mL of lysis buffer provided therein. Cells were sheared by passing through a sterile 60 cc syringe fitted with an 18-21 gauge needle. This process was repeated 4 times. Lysates were incubated at 45° C for 15 min while shaking after which 950 µL of 5M NaCl was added. Upon mixing thoroughly, the solution was passed through the syringe an additional 4 times in order to cause DNA shearing. Seventy-five mg of powdered Oligo (dt) Cellulose was then added and the tube was rocked gently at room temperature for 1 h. Afterwards, lysates were centrifuge at 2000-4000 x g in a table top centrifuge for 5 min, supernatants were discarded, and pellets were washed twice with binding buffer and low

centrifugation. Pellets were dissolved in sterile water and stored until further use.

Degenerate primers were designed and synthesized as disclosed herein. The 5'primers were designed to anneal to the signal peptide of the heavy (SEQ ID NO:16) or light chain genes (SEQ ID NO:17) based on published sequences (Kabat et al., 1987, Sequences of Proteins of Immunological Interest; 4th Ed. U.S. Department of Health and Human Services, Public Health Service, NIH). The 3' primers were designed to anneal to the constant region of the heavy (SEQ ID NO:18) and light chain genes (SEQ ID NO:19).

Genes of interest were obtained by PCR. The reaction (100 µL total volume) was prepared using a PCR Core Kit (Boehringer Mannheim, Corp., Indianapolis, IN) and typically contained 0.5 µg cDNA template and 100 pm of each primer. The heavy and light chain cDNAs were PCR amplified in separate experiments. PCR conditions were as follows: [95° C (1 min); 55° C (0.5 min); 72° C (0.5 min)] for 30 cycles followed by 1 cycle of 95° C (1 min); 55° C (0.5 min); 72° C for 2 min. PCR products were observed using agarose gel electrophoresis and ethidium bromide staining. Amplification of the heavy and light chains resulted in DNA fragments of about 730 bp each entered herein as SEQ ID NO:24 and SEQ ID NO:21, respectively. Products were then digested with the NheI, cloned into pBlueBac1 (Invitrogen, Carlsbad, CA) and transformed into DH5α strain of *E. coli* using standard procedures. Clones were recovered and digested with NheI to verify the DNA insert. Plasmid DNA was produced, isolated, and sequenced as previously described using several external and internal primers. The DNA encoding the light chain (SEQ ID NO:50) was modified as follows: a 5' primer, entered herein as SEQ ID NO:20, was designed to replace the native mouse signal peptide with a

GENERATION OF RECOMBINANT BACULOVIRUS EXPRESSING Mab-TP1

SF9 (Invitrogen) cells were cultured at 27° C in spinner flasks with Sf900II (Gibco, Gaithersburg, MD) serum free complete medium supplemented with penicillin/streptomycin/fungizone at 1/2 rate. Cells were split to 3 x 10^5 cells/mL every four days. SF9 cells were seeded at 8 x 10^5 cells/well in a six well plate and allowed to attach for 1 h at 27° C. The medium was discarded and cells were washed twice with fresh medium without antibiotics. After the last wash, 1.5 mL of fresh medium without antibiotics was added. In a polystyrene tube, 0.1 µg of linearized parental Baculovirus DNA (Baculogold, PharMingen, San Diego, CA) was mixed with 0.5 µg of the transfer vector (AcMP3/TpLCp67 or AcMP3/TpHCp67 plasmid), followed by transfection mix as described in the standard cationic lipisome mediated transfection protocol as provided by CLONTECH (Palo Alto, CA). Transfection medium was harvested after four days and samples of medium and cell pellet were analyzed for protein expression by western analysis as described herein. A 1:1000 dilution of goat anti-mouse FAb-specific IgG conjugated to horseradish peroxidase (Sigma Chemical Co.) was used with a ECL detection kit (Amersham). The results indicated that light chain and heavy chain proteins could be produced and recognized by appropriate antibodies, thus authenticity was confirmed.

To determine if functional antibodies could be produced, recombinant virus, AcMP3/TpLCp67 and AcMP3/TpHCp67 were co-infected in SF9 cells. The cell medium was harvested and used as the primary antibody in Western analysis using precursor Δ-9 desaturase (pDAB432D) as antigen. The expected sized band (42 kDa)

added to the 3' end of the gene and the p67 leader sequence (SEQ ID NO:34) was added at the 5' terminus.

The SCAb-TP1 gene (SEQ ID NO:31) was reconstructed by PCR using the reaction conditions described above. Amplification of DNA according to SEQ ID NO:22 with primers according to SEQ ID NO:35 and SEQ ID NO:36 resulted in a product of ~450 bp, having the sequence entered herein as SEQ ID NO:40. Amplification of DNA according to SEQ ID NO:25 with primers according to SEQ ID NO:37 and SEQ ID NO:38 (TABLE V) resulted in a product of ~440 bp having a DNA sequence according to SEQ ID NO:41. Approximately equal molar amounts of each product (SEQ ID NO:40 and SEQ ID NO:41) were added to another reaction with primers according to SEQ ID NO:35 and SEQ ID NO:38 yielding a product of ~846 bp (SEQ ID NO:42). An additional PCR reaction was performed with DNA according to SEQ ID NO:42 using the primer pair having a DNA sequence according to SEQ ID NO:35 and SEQ ID NO:39 to add a stop codon to the 3' end of the gene. The ~849bp product (SEQ ID NO:43) was isolated from a 1% agarose gel, digested with Xba I/Not I and ligated into the AcMP3 transfer vector (AcMP3/TPSCegt). An appropriate clone was identified and sequenced.

AcMP3/TPSCegt and BacPAK6 Bsu36 I linear parental DNA (Clontech) were co-transfected into SF9 insect cells to create recombinant virus. Cationic lipisome mediated transfection was performed as described in the instructions included with the BacPAK6 DNA. The transfection media was harvested after 48 h and 0.5 mL was used to infect a 175cm^2 flask containing 2 x 10^7 SF9 cells to amplify the recombinant virus. Western analysis performed as described herein identified a ~28kD protein in infected cells using an anti-HIS antibody (Qiagen, Inc., Chatsworth, CA). SCAb-Tp1 produced in the Baculovirus system was compared to the native MAb-Δ-9TP1 in Western blots using E. coli expressed Δ-9 desaturase

GGCGCGCCGC GATCGCTTGC AGATCTGCAT GGG (SEQ ID NO:54). Nucleotides 4808-5416 of pDAB439 comprised the double enhanced 35S promoter, with nucleotides 5070 to 5416 corresponding to nucleotides 7093 to 7439 of the Cauliflower Mosaic Virus genome (Franck et al., (1980) Cell 21:285-294). Nucleotides 4808 to 5061 of pDAB439 were a duplication of nucleotides 5068 to 5321. Nucleotides 5062 to 5067 of pDAB439 comprised the linker CATCGA. Nucleotides 5417-5436 of pDAB439 comprised the linker GGGGACTCTA GAGGATCCAG (SEQ ID NO:55). Nucleotides 5437 to 5547 of pDAB439 corresponded to nucleotides 167 to 277 of the Maize Streak Virus genome (Mullineaux et al., (1984) EMBO J. 3:3063-3068). Nucleotides 5548 to 5764 of pDAB439 corresponded to the modified first intron of the maize alcohol dehydrogenase gene (Adh1-S) (Dennis et al., (1984) Nucleic Acids Res. 12:3983-4000). The modification resulted in removal of 343 nucleotides (bases 1313 to 1656) with bases 1222 to 1312 (intron 5' end) and nucleotides 1657 to 1775 (intron 3' end) of the maize Adh1-S gene remaining. Nucleotides 5765 to 5802 of pDAB439 corresponded to Maize Streak Virus (MSV) nucleotides 278 to 312, followed by the linker sequence CAG. Both sections of the Maize Streak Virus, hereinafter MSV, sequence comprised the untranslated leader of the MSV coat protein V2 gene, and were interrupted in plasmid pDAB439 by the modified Adh1 intron. Nucleotides 5803 to 6359 of plasmid pDAB439 corresponded to nucleotides 29 to 585 of the phosphinotricin acetyl transferase (BAR) gene of Streptomyces hygroscopicus (White et al., (1989) Nucleic Acids Res. 18:1062). To facilitate cloning, nucleotides 34 and 575 of the published sequence were changed from A and G to G and A, respectively. This sequence served as the selectable marker in plant cells. Nucleotides 6360 to 6364 comprised the linker GATCT. Nucleotides 6365 to 6635 of pDAB439 corresponded to nucleotides 4420 to 4683

was removed and the gold particles were resuspended in 1 mL of absolute ethanol. This suspension was diluted with absolute ethanol to obtain 15 mg DNA-coated gold/mL. Approximately 600 mg of embryogenic callus tissue was spread over the surface of Type II callus maintenance medium as described herein lacking casein hydrolysate and L-proline, but supplemented with 0.2 M sorbitol and 0.2 M mannitol as an osmoticum. Following a 4 h pre-treatment, tissue was transferred to culture dishes containing blasting medium (osmotic media solidified with 20 g/L tissue culture agar (JRH Biosciences, Lenexa, KS) instead of 7 g/L GELRITE (Schweizerhall, South Plainfield, NJ). Helium blasting accelerated suspended DNA-coated gold particles towards and into the prepared tissue targets. The device used was an earlier prototype of that described in US Patent #5,141,131 which is incorporated herein by reference. Tissues were covered with a stainless steel screen (104 µm openings) and placed under a partial vacuum of 25 inches of Hg in the device chamber. The DNA-coated gold particles were further diluted 1:1 with absolute ethanol prior to blasting and were accelerated at the callus targets four times using a helium pressure of 1500 psi, with each blast delivering 20 µL of the DNA/gold suspension. Immediately post-blasting, tissue was transferred to osmotic media for a 16-24 h recovery period. Afterwards, the tissue was divided into small pieces and transferred to selection medium (maintenance medium lacking casein hydrolysate and L-proline but having 30 mg/L BASTA (Agrevo)). Every four weeks for 3 months, tissue pieces were non-selectively transferred to fresh selection medium. After 7 weeks and up to 22 weeks, callus sectors found proliferating against a background of growth-inhibited tissue were removed and isolated. The resulting BASTA-resistant tissue was subcultured biweekly onto fresh selection medium. Following gas chromatography/fatty acid methyl

- 10 weeks in the 5 gallon pots. R_1 seed was collected at 40-45 days post-pollination.

Embryogenic callus material containing the genes of interest was maintained as described herein. Continuous production of somatic embryos, which make up a large portion of embryogenic callus, was performed by transferring the callus tissue every two weeks. While the somatic embryos continued to proliferate, they usually remained in an early stage of embryo development because of the continued presence of 2,4-D in the culture medium. Somatic embryos could be regenerated into plantlets when callus was subjected to the regeneration procedure described herein. During regeneration, somatic embryos formed roots and a shoot, subsequently ceasing development as an embryo.

Somatic embryos were made to develop as seed embryos by growing embryogenic callus on MS medium containing 6% (w/v) sucrose. The callus was grown for 7 days and then somatic embryos were individually transferred to MS medium with 6% sucrose and 10 µM abscisic acid, hereinafter ABA.

EXAMPLE 10

SOUTHERN ANALYSIS OF TRANSFORMED CALLUS AND PLANT TISSUES

BASTA resistant lines transformed with various plasmids were characterized by Southern analysis to confirm the presence of the transgene using a DNA probe specific for the coding region of the gene of interest. DNA from leaf material was analyzed.

Leaf material from plants was harvested at the 6-8 leaf stage. Genomic DNA was prepared from lyophilized tissue as described by Saghai-Maroof et. al. ((1984) *Proceed. Nat. Acad. Sci. USA* 81:8014-8018). Eight µg of each DNA was digested with the restriction enzyme(s) specific for each plasmid construct using conditions

the gel and electrophoresed for 3 h at 60 V in 10 mM phosphate buffer. RNA was transferred from the gel to GENESCREEN PLUS membrane (NEN Research Products, Boston, MA) by capillary transfer with sterile water as the transfer medium. Following transfer, the RNA was crosslinked to said membrane by UV (STRATALINKER, Stratagene Cloning Systems, Inc., La Jolla, CA).

The RNA blot was prehybridized for 3 h at 42° C in hybridization buffer (50 mM sodium phosphate, pH 6.5, 0.8 M sodium chloride, 1 M EDTA, 0.2% sodium dodecyl sulfate, 0.05% bovine serum albumin, 0.05% Ficoll Type 40, 10% dextran sulfate). A hybridization probed specific for the antibody made against the transit peptide was labelled with 50 µCi of α-^{32}P-dCTP (NEN Research Products) using READY-TO-GO labelling beads (Pharmacia, Piscataway, NJ) according to the manufacturers instructions and purified over NUCTRAP columns (Stratagene). The labeled probe was denatured by boiling for 5 min, chilled on ice for 5 min, and added directly to the prehybridization blots. Hybridization was done in SEAL-A-MEAL bags (Dazey Corp., Industrial Airport, KA), at 42° C for 16 h. Blots were washed 6 times for 0.5 h each in large excess of prewarmed washing solution (20 mM $NaPO_4$, pH 6.5, 50 mM NaCl, 1 mM EDTA, and 0.1% sodium dodecyl sulfate) at 60° C. The blot was exposed to a phosphor storage screen, scanned on a Molecular Dynamics Personal PHOSPHORIMAGER (Molecular Dynamics, Inc., Sunnyvale, CA) for analysis.

For analysis of R_0 plants, leaf tissue (~ 0.2 g) was harvested from plants transformed with pDAb439/TPE and pDAB439/TPnoE. RNA was extracted, fractionated, blotted to membranes and probed as described above. Lines that were positive for transgenic RNA at the callus stage were found to be positive at the R0 plants stage. These data indicated that the transgene was integrated and expressing mRNA related to the gene of interest.

reduced levels (10 to 50%) of Δ-9 desaturase when compared to control lines. These data indicated a direct correlation between the presence and expression of the transgene and reduced Δ-9 desaturase levels (Table 1).

Table 1. Comparison of Δ-9 desaturase levels in maize lines transformed the pDAB439/TPE and pDAB439/TPnoE relative to control.

Line	SCAb-TP1 Protein	Reduced Δ-9 desaturase
TPE-04	+	yes
TPE-05	+	yes
TPE-06	+	yes
TPE-07	+	yes
TPE-08	nd	yes
TPE-09	+	yes
TPE-10	+	yes
TPnoE-01	nd	yes
TPnoE-04	nd	yes
TPnoE-05	+	yes
TPnoE-07	+	yes
TPnoE-11	+	yes
TPnoE-12	nd	yes

nd: not determined

Fertile plants containing pDAB463/TPnoE were crossed with inbred CQ713 HO-I (Mycogen Seeds, San Diego, CA) as pollen donors and seed were obtained. These seed were planted and molecular analyses were performed on leaf samples to determine the presence of SCAB-TP1 gene, SCAB-TP1 protein and their effect on Δ-9 desaturase levels. Reduction in Δ9-desaturase levels (40-70%) was found to correlated with the presence of the transgene and SCAB-TP1 expressed therefrom.

EXAMPLE 13

Antibody-Mediated Down-Regulation of Maize Palmitoyl-ACP Thioesterase (PTE)

Antibodies directed against the chloroplast transit peptide domain of the PTE protein are used in this

performed in a 6-well plate with 6 x 10^5Sf9 cells/well. BacPAK6 linear DNA (Clontech, Palo Alto, CA) was used as parental DNA in the cotransfection. Bacfectin reagent was used to facilitate cotransfection (included with BacPAK6 DNA). Transfection media was harvested at 3 and 6 days post-transfection. Sf9 cells (6 x 10^5cells/well) were infected with 100µl transfection media. After 3 days, cells were harvested and western blots were performed. Blots were probed with rabbit polyclonal antibody generated against the mature PTE protein or to the transit peptide of the precursor protein (rabbit 1 or rabbit 2). The blot probed with antibody to the mature protein showed 2 distinct bands for PTE with or without the transit peptide. Blots probed with antibody to the transit peptide only detected the higher molecular weight form of the PTE containing the transit peptide.

Production of hybridoma cell lines that produce antibodies that bind to the PTE precursor protein, cloning of the antibody genes and single chain antibody gene, and transformation into corn can be carried out as described in foregoing Examples 5 through 9.

10. The method of Claim 8 or 9 wherein said antibody or fragment thereof is a single chain antibody molecule.

11. The method of any one of Claims 8 to 10 wherein said epitope comprises at least 6 amino acids, said amino acids being continuously adjacent to each other in said transit peptide.

12. A plant cell wherein the steady state level of a passenger protein found therein has been decreased by the method of any one of Claims 8 to 11.

13. A plant or progeny thereof derived from a plant cell of claim 12.

14. A monoclonal antibody that specifically binds to an epitope found in maize stearoyl-ACP Δ-9 desaturase.

15. A monoclonal antibody of claim 14 that specifically binds to an epitope found in the polypeptide of SEQ ID NO:15.

16. An antibody of Claim 15 produced by 10E10, having ATTC Designation HB-12544.

17. A hybridoma cell line designated 10E10, having ATTC Designation HB-12544.

18. A monoclonal antibody that specifically binds to an epitope found in the maize palmitoyl-ACP thioesterase transit peptide.

19. A monoclonal antibody of claim 18 that specifically binds to an epitope found in SEQ ID NO:56.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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